Reference: *Biol. Bull.* **188**: 32–45. (February/March, 1995)

Prespawning Behavior, Spawning, and Development of the Brooding Starfish *Leptasterias polaris*

JEAN-FRANÇOIS HAMEL AND ANNIE MERCIER

Département d'Océanographie, Université du Québec à Rimouski, Centre Océanographique de Rimouski, 310 allée des Ursulines, Rimouski (Québec), Canada G5L 3A1

Abstract. Our study focused on the precise reproductive behavior of the starfish *Leptasterias polaris* (Müller and Troschel) before and during spawning—a subject of much speculation and evident ecological importance. Between the third week of December 1992 and mid-January 1993, we observed spawning in the laboratory that roughly corresponded to field observations in the Lower St. Lawrence Estuary. In experimental tanks provided with natural environmental conditions, the spawning was preceded by 7 to 8 weeks of complex aggregative interactions among the starfish. The individuals, which usually avoid each other, began to make discreet arm contact, which intensified with time and eventually led to the superposition of two or more starfish, independently of sex. The interactions seem to be associated with decreasing temperature, because aggregative and spawning behaviors were not observed under stable temperature conditions. Male spawning is first initiated when the temperature falls to about 2°C during minimum daylength (<9 $h \cdot d^{-1}$). In seawater, the spermatozoa are negatively buoyant and tend to deposit as a sticky film on the substrate, where they enter a state of low activity. Stimulated by male spawning, females spawn on the layer of sperm, which is reactivated by contact with the oocytes, ensuring fertilization. In the laboratory, the fertilized eggs undergo first cleavage in 45 h, become brachiolaria in 40 days, and form fully developed young starfish within 5.5 to 6 months, synchronously with populations in the field. The embryos develop at the same rate even when not brooded, suggesting that the brooding behavior in *L. polaris* serves mainly to keep the eggs clean, healthy, and protected against predation.

Introduction

Successful fertilization constitutes a critical stage in marine invertebrate reproduction, and many organisms develop strategies to maximize this important step (Himmelman, 1981; Giese and Kanatani, 1987). Starfish show diversified reproductive behaviors. In many species, gametes are broadcasted by both sexes, with fertilization in the water being enhanced by synchronization of spawning (Hyman, 1955; Strathmann, 1987; Chia and Walker, 1991). In other starfish, males broadcast spawn in the usual fashion, and females emit fewer gametes but brood their embryos to fully developed young starfish (McClary and Mladenov, 1990; Chia and Walker, 1991). *Leptasterias polaris*, which protects its embryos for 5 to 6 months, is among the few species that brood by overlaying the eggs deposited on the substrate (Emerson, 1977; Himmelman *et al.*, 1982; Boivin *et al.*, 1986). Although brooding starfish are generally small-sized, with lecithotrophic development (Chia and Walker, 1991), *L. polaris* can reach diameters up to 50 cm (Boivin *et al.*, 1986) and are probably among the largest brooders.

Prespawning and spawning behaviors are very important to reproductive success in marine invertebrates. Breeding aggregations have been observed in a number of asteroids (Chia, 1968; Komatsu, 1983; Minchin, 1987; Young *et al.*, 1992; Slattery and Bosch, 1993). Many authors suggest that such aggregations could minimize sperm dilution and increase fertilization success (Ormond *et al.*, 1973; Levitan, 1991; Levitan *et al.*, 1992), as exemplified by the pairing strategies in *Archaster typicus* (Run *et al.*, 1988) and *Neosmilaster georgianus* (Slattery and Bosch, 1993). In those species, the male, after finding a female, mounts her before spawning (Ohshima and Ikeda, 1934; Komatsu, 1983; Run *et al.*, 1988; Slattery and Bosch, 1993). There is also evidence that the spatial distribution of broadcast spawners has a major influence on the probability of fertilization due to gamete viability (Pennington, 1985; Yund, 1990; Levitan *et al.*, 1992; Young *et al.*, 1992). Young *et al.* (1992) suggested that aggregations

Received 7 December 1993; accepted 4 November 1994.

could be useful in overcoming the absence of the usual spawning cues (*e.g.*, light, temperature) in bathyal echinoid populations. The possible role of pheromones and other possible attractants on clustering and related spawning inducement in starfish has been examined (Lewis, 1958; Miller, 1989). Komatsu (1983) suggested that initial heterosexual recognition and pairing in *A. typicus* allow male spawning to be induced by release of mature oocytes from females. However, most studies on aggregation have associated it with cooperative feeding or predation avoidance (Ormond *et al.*, 1973; Blankley and Branch, 1984; Sloan, 1984; Pearse and Cameron, 1991).

Although different kinds of aggregations during spawning have been observed, little is known about the involvement of grouping prior to spawning other than recent work on the bathyal sea urchin *Stylocidaris lineata* (Young *et al.*, 1992). Moreover, prespawning interactions have never been discussed in respect to environmental factors such as photoperiod and temperature, which are known to influence gametogenesis and spawning, respectively (Giese and Pearse, 1974; Himmelman, 1981; Pearse and Walker, 1986; Pearse *et al.*, 1986; Pearse and Cameron, 1991). As for fertilization strategies, observations on gamete interactions, other than mutual recognition and attraction, remain scarce. Sperm motility and respiration activation by egg extracts have been studied in sea urchins (Suzuki *et al.*, 1982) and in horseshoe crabs (Clapper and Epel, 1981), but only sperm chemotaxis has been described in detail for starfish (Miller, 1985).

The starfish *Leptasterias polaris* can be kept in laboratory facilities that reproduce natural conditions, and this has provided a chance to record and describe its aggregative behavior both before and during spawning. Further evidence from experiments on gamete behavior and embryonic development allowed us to better understand the evolutionary strategy that seems to link spawning, gamete fertilization, and brooding activities in this species.

Materials and Methods

Using scuba, we collected 60 specimens of *Leptasterias polaris* from a depth of about 10 m on the south shore of the Lower St. Lawrence Estuary (48° 21′ N; 68° 47′ W), eastern Canada. The animals, ranging from 150 to 200 mm in diameter, were collected in May 1992, to ensure that they were acclimatized well before the December spawning that we expected on the basis of previous observations by Boivin *et al.* (1986). The starfish were kept in tanks to which seawater from the collect site was supplied by a flow-through system and light on natural photoperiod was provided through large windows. Physical and chemical conditions were therefore similar to the natural environment. Preliminary determination of sex in *L. polaris* demonstrated a natural sex ratio close to 1:1, and this was carefully reproduced in the tanks. To establish the importance of environmental factors on prespawning aggregative behavior, spawning, and development, the temperature and the salinity of the circulating water were continuously recorded. The data for the daylength were provided by the Canadian government (Environment Canada; Atmospheric Environmental Service, Quebec airport). The starfish were given an unlimited quantity of mussels (*Mytilus edulis*, ≈20 mm in shell length), their favorite prey (Himmelman and Dutil, 1991). Frequently very abundant in subtidal environments (Himmelman and Dutil, 1991), *L. polaris* adapts extremely well to experimental conditions.

Prespawning behavior

Starfish behavior was recorded on a regular basis between 4 and 14 times a week depending on activities observed, from November 1992 to February 1993, with complementary observations before and after this period. The number of individuals preying on the mussels, resting on the bottom, and climbing on the sides of the tank were noted. The number of starfish in contact was recorded and categorized as light, when arms touched from the middle part to the tip; intimate, when the arms intertwined for more then half their length; or superposition, when the individuals overlaid one another (Fig. 1). Particular attention was given to reactions of the starfish after renewal of food supply, about twice a month. Data were subsequently combined for weekly comparison of contact intensities. Two control groups were also observed during all prespawning and spawning experiments. Group 1 was maintained under constant conditions in the Quebec Aquarium at a temperature of 6°C, a salinity of 28‰ and a daylength of 10 h; group 2 was kept in continuous darkness with natural conditions of salinity and temperature.

Spawning behavior

As the spawning period approached (Boivin *et al.*, 1986), observations focused on detecting gamete release in relation to the postures adopted by the starfish and to the prevailing environmental conditions. When observed, spawning events were carefully described.

To test the hypothesis that sperm induces spawning in females, a solution (1.2×10^3 spermatozoa $\cdot$ ml^{-1} determined with a hemacytometer under a light microscope) was prepared with freshly collected sperm from a single male. This sperm solution was poured into a tank (4 m^3) among mature females, and their subsequent behavior was recorded. This procedure was carried out four times at different periods under the conditions previously described for the starfish in control group 1. Male starfish were exposed to the same sperm concentrations to see if they would be induced to spawn.

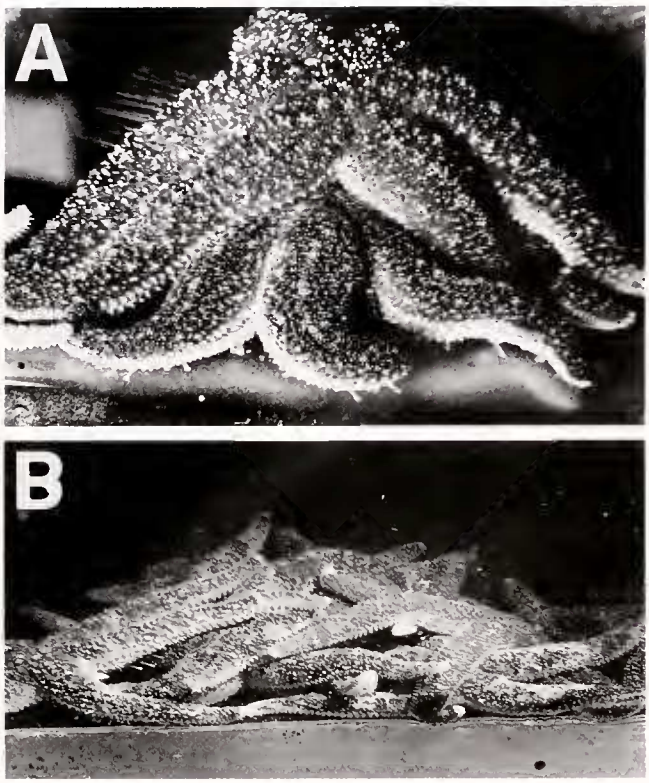

Figure 1. Photographs illustrating the prespawning aggregations of *Leptasterias polaris* in the laboratory. (A) Superposition of two individuals. (B) Massive aggregation of starfish.

Sperm behavior

The term dry sperm refers to undiluted, freshly removed sperm from the mature gonads of at least two males. Activated oocytes were obtained by spreading freshly removed female gonads (including gonoducts) in a petri dish filled with 5 ml of 1-methyladenine 10^{-5} *M*, yielding naturally spawned oocytes after about 45 min of exposure. The buoyancy, capacity for adherence to the substrate, and motility of the sperm were used to describe its behavior in seawater. All experiments on sperm behavior were conducted at a temperature close to the one recorded during the spawning period (2–4°C). All live observations of sperm samples were made under a light microscope (100–400×) and the sperm was kept cool by surrounding the slide with ice cubes. The speed of the spermatozoa was evaluated with a graduated lens, by calculating the number of bars traveled per second. Verifications were undertaken to discard any behavior induced by the light of the microscope or false sperm velocity induced by the sperm-glass interaction (thigmotaxis).

For more than 7 h we continuously recorded the speed, orientation, and flagellar activity of sperm before and after the samples were diluted in seawater (150 μl of dry sperm in 1 l). To examine the behavior of sperm in the water column and on the bottom, the protocol was carried out in both still and agitated (current of 7 $cm \cdot s^{-1}$) water. Samples were taken from the bottom of the beaker and at a middle depth every minute for 15 min, then every 15 min for 7 h, and finally at 24-h intervals until the spermatozoa were dead. This protocol, which was repeated twice using repetitive independent measurements, also allowed the estimation of the sperm concentration, with the proportion of resting and agglomerated sperm in the water and on the bottom over time. To estimate the maintenance of sperm potency, samples of sperm deposited on the bottom of the beaker were collected just after the sperm was released in the seawater and regularly over 72 h. The samples were tested on two replicates of 5–10 freshly activated oocytes. Fertilization success was determined by staining the eggs with the DNA-specific fluorescent dye Hoechst 33258. Using a Leitz Diaplan fluorescence microscope, we determined the proportion of eggs showing a male pronucleus.

After spermatozoa reached a state of low activity (>80% barely moved) in the beaker of the above experiment, we tried to stimulate the sperm by exposing it to oocytes. In separate trials, a sample of sperm collected on the bottom of the beaker was exposed to oocytes of different levels of maturity and of different origins. The conspecific previtellogenic and mature oocytes, classified according to the studies of Boivin *et al.* (1986) and Mercier *et al.* (1994), were first assayed. In the case of mature oocytes we tried both activated oocytes (showing germinal vesicle breakdown) and surgically removed unactivated ones. The activated oocytes of *Asterias vulgaris,* another species of starfish, were also tested for stimulation of *Leptasterias polaris* sperm. The oocytes of both species were routinely washed in filtered seawater and immediately used for the reactivation experiments. The speed and the flagellar activity of sperm were noted every 10 min for the first hour, then periodically until no movement was detectable, using replicates and going through the whole protocol twice. A control sample with no oocytes was tested in the same manner.

To investigate sperm sinking, dry sperm was diluted in seawater (1:60) to a concentration of 120×10^3 spermatozoa $\cdot ml^{-1}$. Five homogeneous replicates (10 μl) of this solution were prepared from eight males. The samples were deposited at a middle depth in a 1000-ml beaker filled with seawater, and the time needed for about 50% of the sperm (in visible filaments) to reach the bottom was recorded. These times were compared to those of sperm collected from three species in which fertilization occurs in the water column, the starfish *Asterias vulgaris,* the sea urchin *Strongylocentrotus droebachiensis,* and the sea cucumber *Cucumaria frondosa.*

To examine sperm dispersion over time, we deposited dry sperm on the bottom of a large dish filled with 1000 ml

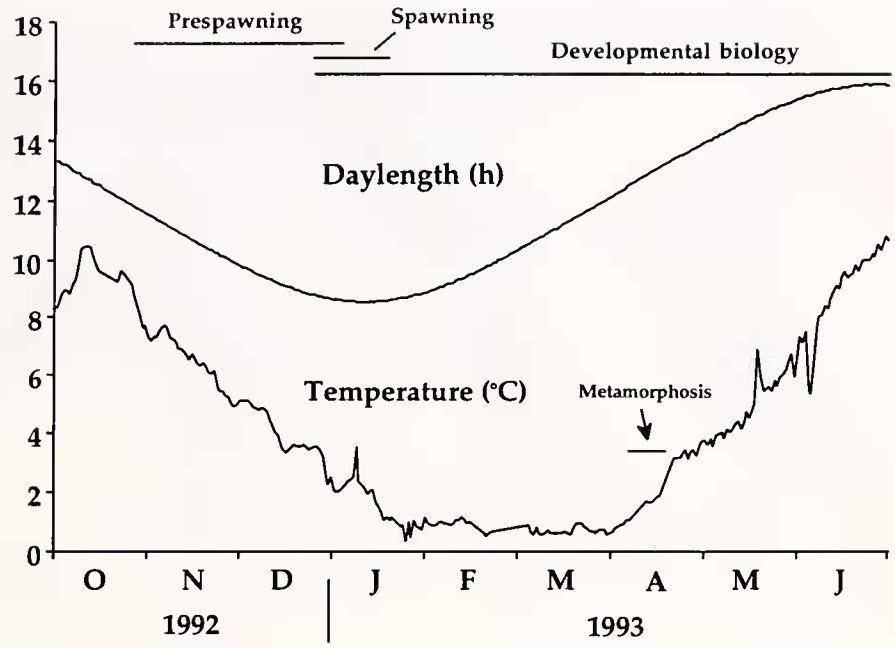

Figure 2. Variation of environmental factors during the reproductive season of *Leptasterias polaris* in the experimental tanks. The arrow points to the beginning of embryo metamorphosis.

of seawater and periodically collected water samples at the surface and on the sides of the dish. The spermatozoan concentrations of the samples were evaluated with a hemacytometer under a light microscope. This experiment was simultaneously performed on live and dead dry sperm of *Leptasterias polaris* and on dry sperm from *Asterias vulgaris, Strongylocentrotus droebachiensis,* and *Cucumaria frondosa.* Sperm longevity could thus be compared for those species under the same conditions.

Development

Whenever we discovered a naturally spawned egg mass, whether brooded or not, it was left undisturbed in the tank so that we could examine development under natural variations of environmental factors. Nonbrooded egg masses were kept clean by periodically agitating the water. Samples were regularly collected with pipettes, from fertilization to young starfish stage, and transferred to 4% formaldehyde/seawater for later examination with light microscopy. These embryos also served to determine developmental kinetics and growth. During the first hour, samples were collected every 2–5 min, then about every day until the brachiolaria stage, and finally once a week. A new stage was considered attained when 50–60% of the embryos reached it. Maximum embryo diameters were measured under a light microscope equipped with a graduated lens.

Results

Prespawning behavior

During summer and early fall, the well-fed starfish clearly avoided each other. Contacts among starfish began in mid-October, coinciding with the first significant decrease of temperature (Fig. 2). The proportion of contact-free starfish decreased, reaching a minimum plateau between 1 November and 15 December (Fig. 3) when temperatures fell to about 4°C. In that same interval, the proportion of starfish involved in intimate contacts increased progressively, with the maximum recorded in the last week of November. Superposition became more frequent in early December and was observed most often just before the main spawning period of late December (Figs. 1, 3) when the water temperature fluctuated between 3° and 4°C. When the temperature fell below 3°C (end of December), the number of superpositions and intimate contacts decreased while spawning was recorded (Fig. 3). Superposition behavior ceased after January and most individuals resumed avoiding one another (Fig. 3), with contact-free starfish accounting for more than 85% of the observed individuals. Only some light contacts and a few intimate ones were observed. No particular sex-specific patterns were noticed for the aggregations, which involved both males and females. Addition of prey to the experimental tanks always provoked a migration of the starfish toward the food source, except 1 week before spawning

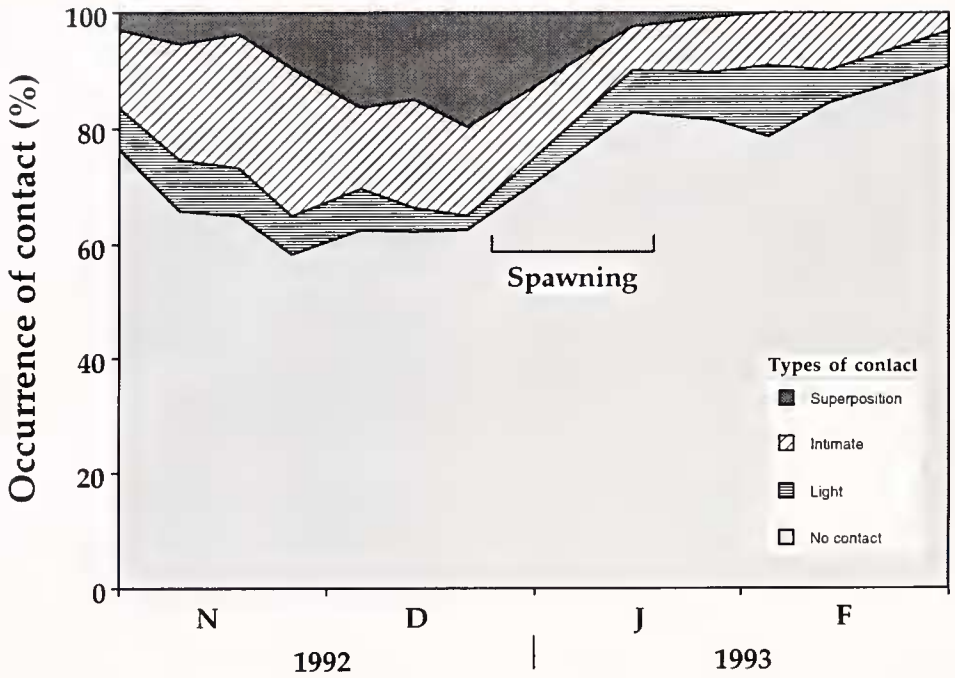

Figure 3. *Leptasterias polaris* Temporal evolution of the prespawning aggregative behavior recorded several times a week for a 3-month period. For each date, the percentage of individuals involved in a particular type of contact was recorded.

when feeding did not disturb the contact behavior between the individuals.

Environmental factors seem to be involved in the initiation and development of the prespawning aggregative behaviors. No aggregation occurred among individuals maintained at constant temperature and photoperiod, but grouping did take place among individuals kept in total darkness with natural temperature.

Spawning

Our experimental information includes actual observations of spawning events in 4 females and 3 males and additional indications from sperm agglutinates on many males and on the substrate. We also observed more than 20 brooding females, which were always discovered within 24 h of spawning. We examined the correlations of all our observations with environmental factors (Fig. 2), which were similar in the laboratory and in the field. Spawning occurred in our tanks from 19 December to 12 January, which roughly corresponded to the period when we observed spawning in the field. During spawning events, the starfish stayed close together, although there was a net decrease in frequency of contact (Fig. 3). The spawning individuals were not paired or superposed.

Spawning events. Figure 4 schematically illustrates the spawning behavior we observed in the experimental tanks. When a male spawned, it elevated the central disk, standing on the curved tip of its arms, and emitted sperm as a whitish stream from the six interradial aboral gonopores. Emission continued for more than an hour. Qualitative observations showed the sperm to be negatively buoyant, with a tendency to deposit on the substrate. The first female spawning was observed after male spawning. The female remained flat on the substrate to release eggs while its arms were extended, then progressively adopted the characteristic brooding posture in "pinwheel" shape (Figs. 4, 5a, b). The average 300–500 spawned oocytes emerged individually at a rate of about 1 oocyte every 2–5 s from each of the six aboral gonopores located between the arms. Almost all the spawning starfish observed were on a vertical substrate (aquarium wall or rock face). The eggs had a tendency to fall and were retained by the ambulacral podia and the curved arms of the female (Fig. 5c). A number of eggs (possibly 50%) were, however, lost before the end of a given spawning event. The final posture adopted by the female was not always a perfect pinwheel shape but seemed designed to cover the egg mass (around 20–25 eggs $\cdot$ cm^{-2}) in the best way possible. Consequently, some brooding individuals were seen with two or three arms extended somewhat to cover isolated groups of oocytes.

Induction of spawning. Although the first individuals to spawn were males, spawning events were subsequently recorded in both sexes alternately throughout the spawning period (Fig. 2). Nevertheless, some correlations con-

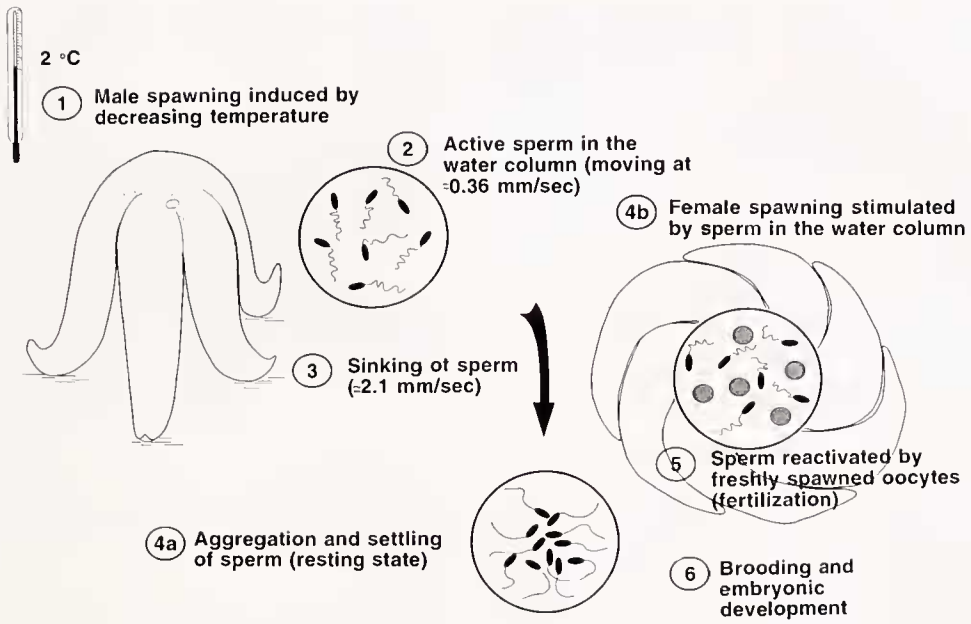

Figure 4. Schematic illustration of spawning behavior for male and female *Leptasterias polaris*, showing the relation between them.

cerning the induction of spawning could be made in the course of our experiments. A few isolated male spawnings occurred during minimum daylength (≤9 h) when water temperature was around 2°–3°C (first sight of sperm filaments) in late December (Fig. 2), but most spawnings were observed in January as the temperature fell further. The temperature fluctuated between 2° and 4°C (Fig. 2) throughout the following weeks of spawning, and gamete release seemed to be closely related to these variations. The same spawning pattern was observed in control group 2, maintained in natural temperature and total darkness, whereas no spawning occurred in control group 1, kept at steady temperature and photoperiod. As a result of the seasonally low primary production, the tanks provided with seawater from the estuary, where spawnings were recorded, contained virtually no phytoplankton. Salinity continuously fluctuated between 26 and 32‰ without any consistent increasing or decreasing trends (data not presented). During qualitative observations, the presence of sperm filaments seemed to be correlated with subsequent female spawning within a few hours. Complementary experiments conducted in replicates showed that the introduction of sperm in the water induced spawning of several females in the controlled environment (control group 1), therefore minimizing the importance of temperature in female spawning. No male spawning was induced by the presence of sperm.

Sperm behavior

A microscopic examination of the sperm showed that the head (more or less spherical) measured 3.25 ± 0.25 μm and the flagellum 62 ± 3 μm. The negative buoyancy of the male spawn caused it to sink (mainly as white filaments), at a rate of about 2.1 mm · s^{-1}. Only a small portion was resuspended after reaching the bottom. We examined the motility of sperm artificially maintained in the water column compared to the motility of sperm settled on the bottom (Fig. 6). Freshly extracted dry sperm contained nonmotile spermatozoa. Upon introduction to the seawater, the spermatozoa immediately displayed a major increase of activity, both in the water and on the bottom (Fig. 6). Within the first 30 min of water contact, 100% of the spermatozoa had reached a velocity of 250–350 μm · s^{-1} and showed intense flagellar activity, resembling a helical movement with 6–7 revolutions · s^{-1}. The spermatozoa maintained in suspension continued to show the same high velocity and activity with no marked net decrease for the whole 425 min of observation. In contrast, after reaching a maximum velocity (after 40–50 min) in synchrony with the sperm in suspension, the sperm on the bottom showed an abrupt decrease of activity (reduced velocity and flagellar movement). The settled spermatozoa attained a low velocity (≈50 μm · s^{-1}) after 120 min of contact with seawater (Fig. 6a). This corresponded to an increase in the inactive population of spermatozoa, which rose from 7% to 56% in the same period (Fig. 6a). The spermatozoa seemed to gradually reach a state of almost null velocity (about 0–15 μm · s^{-1}) in which they only quivered and moved by a wave along the flagellum (proximal to distal) with a 10° angle. The percentage of settled spermatozoa that attained a state of low activity was 47%

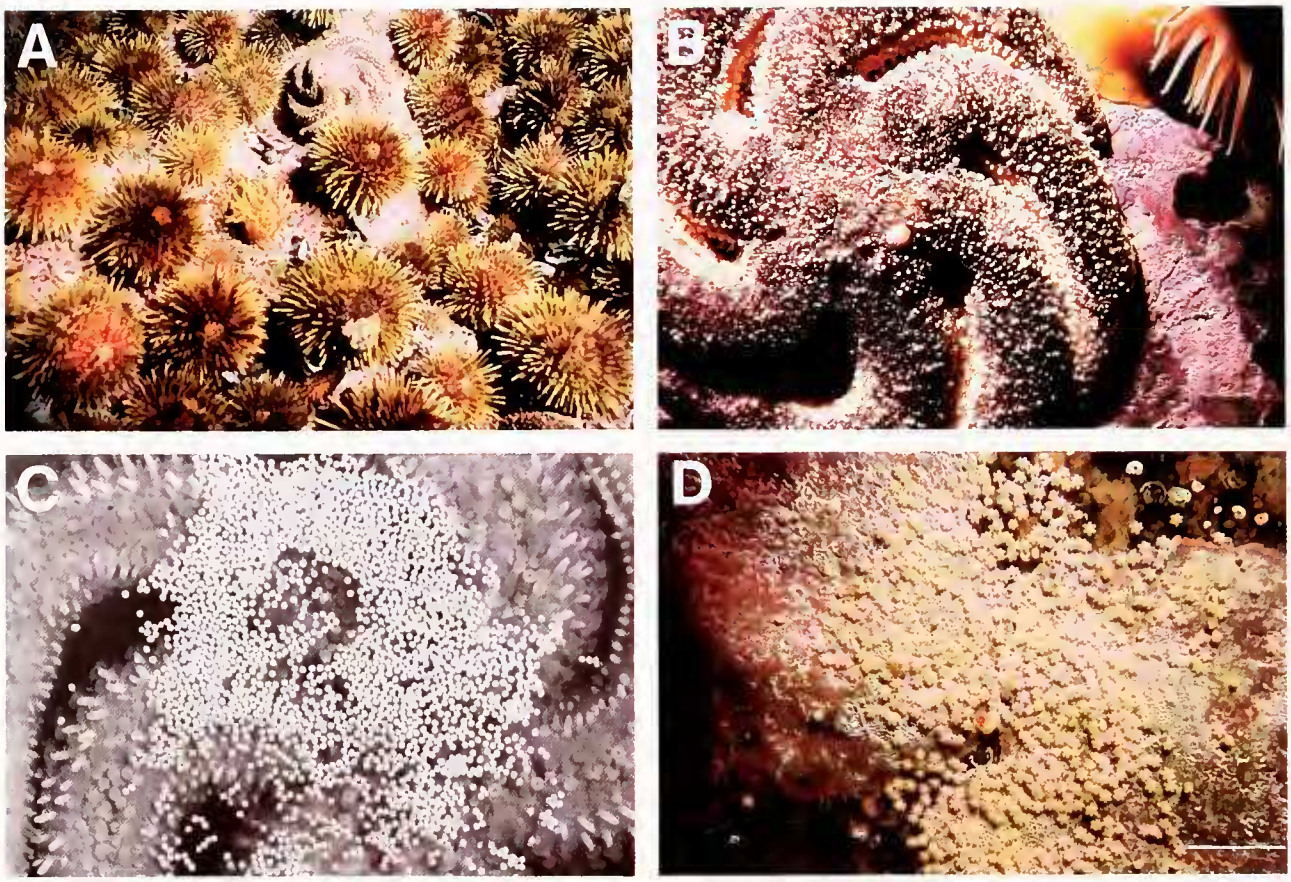

Figure 5. Underwater photographs showing (A) *Leptasterias polaris* brooding in its natural habitat surrounded by sea urchins; (B) close-up of a brooding female on the rocky bottom at 30 m depth; (C) fertilized eggs under a female; (D) young starfish after about 5.5 months of growth in their natural habitat (the female was previously removed). The scale bar represents 15 mm and applies to photographs (C) and (D).

after 120 min of contact with seawater, 62% after 250 min, and a maximum of 80% after 380 min (Fig. 6a). On the bottom and most probably on the glass walls, the increased number of nearly inactive spermatozoa was responsible for the decrease in overall velocity of the population. This correlation could also be made for the sperm in the water column, although the small apparent decrease in velocity could only be visually associated with the slight increase in inactive spermatozoa (Fig. 6b). Another progressive phenomenon was the formation of sperm conglomerates (dense aggregations), which began about 180 min after the sperm came in contact with seawater and reached a maximum (100 and more spermatozoa together) after 380 min. About 8 h after entry into still seawater, the sperm covered the bottom and glass walls of the dish. This also occurred in agitated conditions, although after a longer period, showing that the sperm of *Leptasterias polaris* is very adhesive.

The sperm behavior of *Leptasterias polaris* differed from that observed in the other species of echinoderms tested: the sperm of *Asterias vulgaris* was diluted within 2 min, before reaching bottom, and those of *Cucumaria frondosa* and *Strongylocentrotus droebachiensis* sank at 1 $mm \cdot s^{-1}$ and 1.5 $mm \cdot s^{-1}$ respectively. The sperm of these species did not adhere to the bottom, but was immediately and almost totally redispersed, becoming well dispersed in seawater within 80 min. In contrast, the majority of *L. polaris* sperm stayed on the bottom until death.

After reaching a state of almost null activity (conglomerated or not), the sperm of *Leptasterias polaris* could be reactivated by contact with conspecific activated mature oocytes (Fig. 7). Within 10 min of exposure to these oocytes, sperm motility was reinitiated. Spermatozoa velocity increased significantly, by 485% ($P < 0.01$, Student's t test), after 20 min of contact, and attained a peak of 230 $\mu m \cdot s^{-1}$ after 50 min. This was almost 12 times the original speed (Fig. 7). Subsequently, the sperm velocity decreased progressively and reached a minimum after 1020 min (Fig. 7). Reactivation was unsuccessful with unactivated conspecific mature and previtellogenic oo-

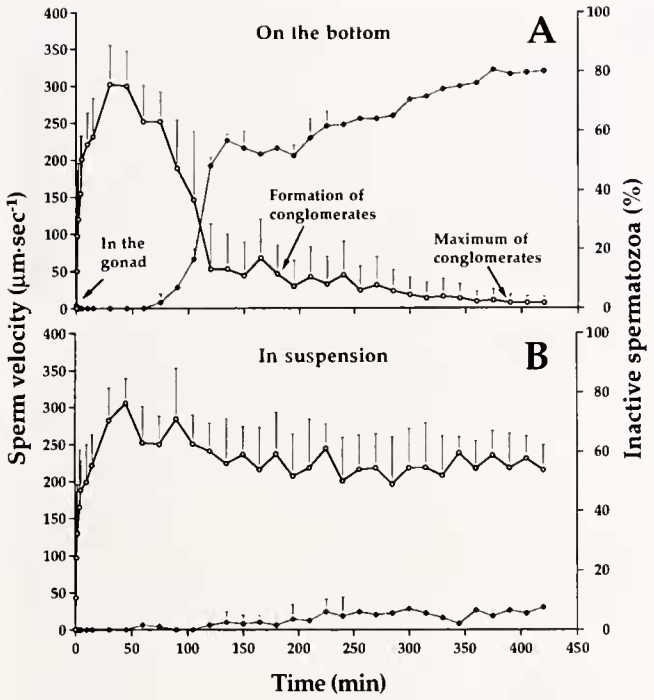

Figure 6. *Leptasterias polaris.* Temporal changes in velocity and activity of sperm settled on the bottom and kept in suspension. The sperm velocity (— ○ —) upon contact with seawater is given with the corresponding percentage of inactive spermatozoa (· · · ◆ · · ·). The error bars represent the confidence intervals (95%).

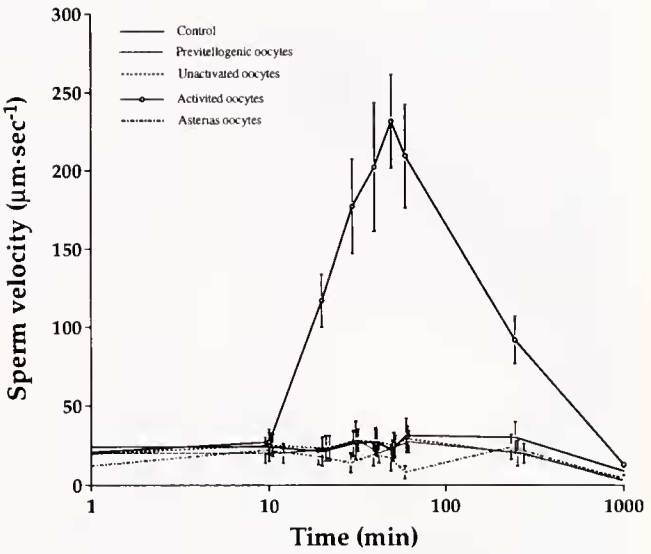

Figure 7. *Leptasterias polaris.* Influence of oocyte maturity on sperm reactivation. The horizontal axis has a $\log_{10}$ scale and the error bars represent the confidence intervals (95%).

cytes as well as with mature activated oocytes of *Asterias vulgaris*. The spermatozoa exposed to those oocytes showed a constant low velocity, comparable to that observed in the unexposed sperm serving as control (Fig. 7).

The sperm of *Leptasterias polaris* was more resistant than that of other species. Most spermatozoa collected from other echinoderms were dead after 8–20 h, whereas those of *L. polaris* remained capable of high fertilization success (57%) for as long as 34 h in seawater (Fig. 8) and showed a high percentage of mortality only after 6–7 days.

Development

Early development. The complete chronology of embryonic development of *Leptasterias polaris* is presented in Table I and Figure 9. The large unfertilized mature oocytes (≈0.85 mm in diameter) were mainly spherical and yellowish, or occasionally light orange. They were covered with a rather thick outer membrane (average of 7.04 µm). After their fertilization, the eggs were attached to one another by the fertilization membrane, showing that the membrane was sticky, especially after reaching the 2-cell stage (Fig. 9a). All the cleavages were of the radial holoblastic type. Later in its development, from the blastula to young gastrula, the embryo decreased in size (≈6%) due to blastomere compaction (Table I). The furrows became shallower on the pole, but tended to deepen on the other pole, eventually forming the blastopore. After 711 h, the embryo's surface became uniform and ciliated. The embryo then began to spin inside the fertilization membrane and hatched 96 h later (Fig. 9e).

Many females moved away from their brood within a week, leaving their embryos uncared for in the experimental tank. No significant difference was observed be-

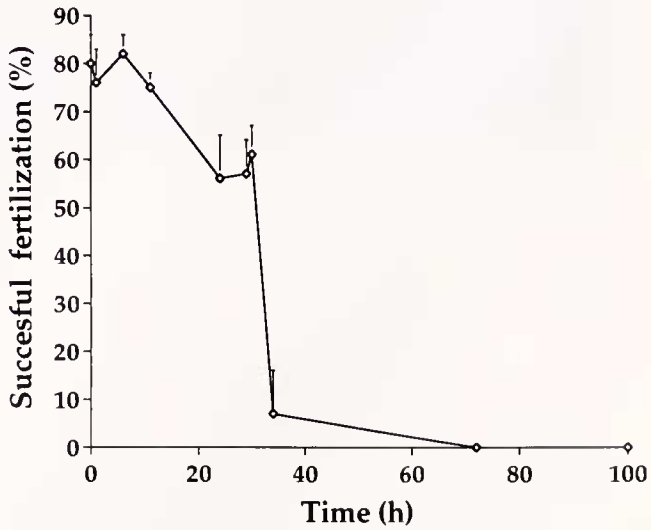

Figure 8. *Leptasterias polaris.* Sperm effectiveness to fertilize activated mature oocytes over time. The error bars represent the confidence intervals (95%).

Table I

Leptasterias polaris: *Kinetics of embryonic development in experimental tanks supplied by running seawater*

Developmental Stages	Time	Size (μm)
Spawning	0	852 ± 36
Early stage of the fertilization membrane elevation	12 min	840 ± 38
Fertilization membrane completely elevated	27 min	940 ± 48
Emission of the first polar body	45 min	939 ± 26
Second polar body	?	?
2-cell	45 h	1079 ± 15
4-cell	86 h	1032 ± 15
8-cell	92 h	1046 ± 12
16-cell	106 h (4 d)	1031 ± 18
32-cell	121 h (5 d)	1062 ± 9
64-cell	133 h (5–6 d)	1103 ± 62
128-cell	146 h (6.1 d)	1080 ± 23
256-cell	156 h (6.5 d)	1101 ± 40
Blastula (compaction)	209 h (8–9 d)	1120 ± 51
Wrinkled-blastula	260 h (10–13 d)	1144 ± 45
Young gastrula	493 h (20–21 d)	1056 ± 31
Late gastrula	666 h (27–28 d)	1288 ± 52
Spinning	711 h (29–30 d)	1375 ± 27
Hatching	807 h (33–34 d)	1121 ± 42
Brachiolaria	38–84 d	1190 ± 31
Metamorphosis	75–90 d	1207 ± 70
Young starfish (2 pairs of ambulacral podia/arm)	120–132 d	1348 ± 53
Young starfish (preoral lobe disappears and ocelli are present)	150–170 d	1534 ± 77
Free-moving starfish (visible pyloric caeca and opening of buccal cavity)	180–195 d	2102 ± 102
Small starfish (uprighting movements)	more than 200 d	over 2500

A new stage was considered attained when 50%–60% of the embryos reached it. The standard deviations about the mean size are given.

tween the developmental rate of brooded and nonbrooded embryos during early development up to hatching ($P = 0.392$, Student's t test), which is the latest brooded stage we observed in the laboratory (Table II).

Late development. After the loss of the fertilization membrane upon hatching, the unciliated portion of the late gastrula enabled it to attach to the substrate, well before the appearance of the brachiolar arms (Fig. 9f). The hatched larvae immediately settled on the bottom; however, many embryos were lost by the female at this time of development (especially for those brooding on vertical surfaces). With the growth of the embryo, the arms elongated, becoming very distinct from the dorsal ciliated bulb (larval body), and served the purpose of adhesion to the substrate. Cilia were still present, so the fixation was mainly with the sticky ramified tips that had developed at the end of each arm (Fig. 9g). A depression began to grow in the central portion delimited by the arms (fixing disk), and was used for later fixation on the substrate with the brachiolar arms. The brachiolar stage was prolonged as long as the water temperature remained around 1°C (February and March; Fig. 2), until a sudden warming coincided with metamorphosis (day 75–90). Although there was a concurrent elevated photoperiod, this factor had been increasing for months and cannot be the deciding inducer (Fig. 2). About 50% of the embryos died during the gradual metamorphosis, which was completed around mid-May following the complete disappearance of the brachiolar arms (Fig. 9h–j). At this stage the characteristic yellow color had been lost and the embryo was whitish or translucent, indicating that a large amount of vitelline reserves had been consumed. One month later (mid-June), the young starfish possessed a well-developed buccal cavity, stomach, and pyloric caeca, which were easily observed across the transparent body wall on the oral surface (Fig. 9l) with the madreporite and anus on the aboral surface. The ambulacral podia, baring suckers, became effective in helping the uprighting movement and displacement of the growing starfish, which were capable of coordinated locomotion. Having the capacity to feed and move on their own, the young were self-sufficient about 6 months after fertilization (Figs. 5d, 9k, l).

Discussion

Temporary aggregative behavior is common among marine invertebrates. It has been observed in echinoderms such as echinoids (Pearse and Cameron, 1991; Levitan *et al.*, 1992; Young *et al.*, 1992), ophiuroids (Warner, 1979) and asteroids (Ormond *et al.*, 1973; Sloan, 1980, 1984; Blankley and Branch, 1984; Run *et al.*, 1988) as well as in crustaceans (Gherardi and Vannini, 1993). Those studies have proposed many hypotheses about the meaning and usefulness of aggregation, but only a few have discussed a relationship with reproduction and spawning.

Most of the few reports of aggregation related to reproduction in echinoderms refer to aggregative spawning events in the field (Hendler and Meyer, 1982; McEuen, 1988; Pearse *et al.*, 1988). Pseudocopulation or pairing has been observed in *Archaster typicus* (Ohshima and Ikeda, 1934; Komatsu, 1983; Run *et al.*, 1988) and in *Neosmilaster georgianus* (Slattery and Bosch, 1993), but no such behavior could be detected during our study. Like Chia (1968) in observations of *Leptasterias hexactis*, we noticed that the groupings occurred only as the breeding season approached. Grouping behavior initiated well before spawning, such as observed in *L. polaris*, has not been often reported. Young *et al.* (1992) observed this behavior in the bathyal sea urchin *Stylocidaris lineata*, in which the individuals aggregate during autumn before spawning. Orton (1914) and Lewis (1958) also mentioned

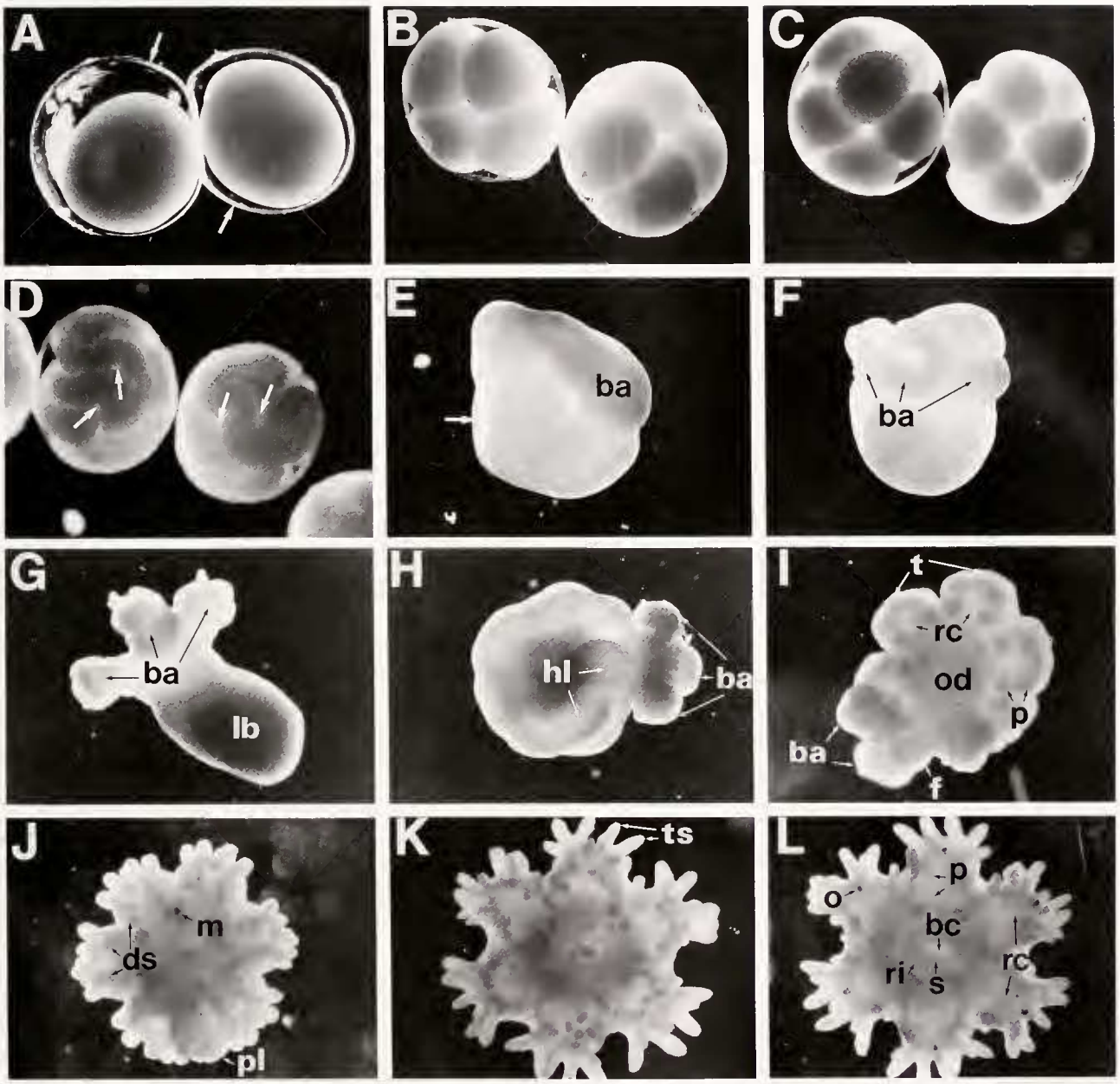

Figure 9. *Leptasterias polaris* Photographic sequence showing the principal steps of development from fertilization to young starfish (4×); see Table I for corresponding age and size of the embryos. (A) Fertilized eggs with fertilization membrane completely elevated (arrows). (B) 4-cell stage. (C) 8-cell stage. (D) Wrinkled-blastula stage on which it is possible to observe the furrows (arrows). (E) Early brachiolaria stage (newly hatched) showing early shaping of brachiolar arms (ba) and the persistent blastopore (arrow). (F) Growth of the three brachiolar arms (ba). (G) Fully developed brachiolarian embryo with the larval body (lb) and the slightly ramified brachiolar arms (ba). (H) Metamorphosing embryo with brachiolar arms (ba), showing the development of the first five hydrocoelic lobes (hl). (I) Further metamorphosed embryo with two pairs of ambulacral podia (p) and a terminal tentacle (t) on each of the five arms. The six hydrocoelic lobes are transforming into the radial canals (rc). A distinct oral disk (od) and the residual brachiolar arms (ba) with central fixing disk (f) are visible. (J) Aboral view of a free-moving, six-rayed young starfish showing clearly visible dorsal spines (ds), madreporite (m), and regressing preoral lobe (pl). (K) Aboral view of a small starfish showing the well-developed terminal spines (ts). (L) Oral view of a small starfish ready to leave the brood. The buccal cavity (bc) has opened and the stomach (s) is visible surrounded by the ring canal (ri). We can also see the ambulacral podia (p), the well-developed radial canals (rc), and the ocelli (o).

the occurrence of this prespawning aggregative behavior in echinoids.

What cues make the starfish come together and why do they display this behavior? Pheromones have often been proposed as the proximate cause (Kanatani and Shirai, 1968), principally acting to synchronize the liberation of gametes (Ormond *et al.*, 1973; Young *et al.*, 1992; Slattery and Bosch, 1993). For broadcast spawners, aggregation and synchronous gamete release appear to be important in minimizing gamete dispersion, ensuring good fertilization success (Levitan, 1988; Levitan *et al.*, 1992). For a protective brooder like *Leptasterias polaris*, synchronous spawning would not be very advantageous as the eggs laid by the female are maintained under the body at all times. Therefore, aggregation is more likely to be related to preparatory recognition. Contact chemoreception is suggested to be a strong sensory stimulus in asteroids (Sloan and Campbell, 1982), maybe to ensure adequate recognition of males and females before spawning. In *Archaster typicus* and *Neosmilaster georgianus*, this recognition is of prime importance because fertilization is ensured by the close superposition of a male and a female (Run *et al.*, 1988; Slattery and Bosch, 1993). In *L. polaris*, our results demonstrate a totally different pattern, in which the prolonged intimate contacts could be related to the chemical induction of synchronized gamete development as demonstrated in sea cucumbers *Cucumaria frondosa* (Hamel *et al.*, unpub. manuscript).

The initiation of aggregative behaviors in *Leptasterias polaris* appears to be correlated with decreasing temperature. Aggregations were observed among all starfish that were supplied with natural seawater and exposed to seasonal changes of water temperature, both when maintained in darkness and when exposed to natural photoperiod. Lower temperatures possibly trigger or enable the liberation of hormones through a pathway otherwise suppressed and may favor the formation of clumps of starfish. This would assure that a fairly good proportion of male and female individuals would be close together and ready to release their gametes during the winter spawning events. We saw no evidence of recognition between sexes but can assume that the male/female ratio close to equality ensures a 50% chance of random heterosexual encounters. Young *et al.* (1992) found that individuals of *Stylocidaris lineata* also aggregated without regard to sex. The spawning events were not synchronized—as the male began liberating sperm before any female spawning could be detected—which differs from spawning sequences mentioned for *Hymenaster membranaceus* (Pain *et al.*, 1982) and for *Archaster typicus* (Run *et al.*, 1988). Male spawning seemed to be triggered when the falling temperature reached about 2°C, an inducer previously suggested by O'Brien (1976) for *L. littoralis*.

The sperm behavior of *Leptasterias polaris* possibly further explains the need for aggregation. Upon its release, a portion of sperm can be dispersed by currents and remain active as long as it is maintained in the water column (Fig. 6), potentially limiting the genetic isolation in a population. From the spawnings successfully induced by sperm in our experimental tanks, we infer that this active fraction is the stimulus for females to spawn. Sperm as a stimulus of spawning has also been discussed by Starr *et al.* (1990) for the sea urchin *Strongylocentrotus droebachiensis*. In fact, sperm suspension in seawater is suggested to be the spawning inducer in many species of ophiuroids and echinoids (Thorson, 1950; Lewis, 1958). The lack of strong epidemic spawnings during natural breeding activities in our tanks could be explained by fluctuations of water temperature around the 2°C threshold at that time (Fig. 2). A few male spawnings could have been triggered in late December, then delayed by the rising temperature (to almost 4°C) before being induced again in the second week of January. Females followed this scattered pattern because they probably need to be close to a sperm source for spawning to be induced. The negative buoyancy and stickiness of sperm causes most spermatozoa to settle on the substrate where they gradually enter an inactive state. The settling ensures a minimal dispersion of sperm but makes fertilization dependant on the proximity of individuals, which is achieved by aggregation. Further, sperm inactivation seems an effective energy-saving behavior, extending the viability of settled sperm up to 6 or 7 days, which is much longer than the 2- to 3-day longevity of sperm maintained in the water column. The extreme endurance of *L. polaris* sperm is further emphasised upon comparison with that of the other asteroids, holothuroids, and echinoids tested, for which the spermatozoa did not survive longer than a day at the temperature normally recorded during their spawning periods. These short-lived spermatozoa display a dispersion behavior that we could probably associate with organisms having synchronously spawning males and females. The longevity of sperm from *L. polaris* is probably an advantage given the asynchronous spawning of the sexes in this species.

We could not determine whether females were attracted by deposited sperm, but the delay between male and female spawnings seems advantageous. Because the spermatozoa are present on the medium before eggs are emitted, they do not have to overcome the protective barrier maintained by the brooder. Thanks to its adhesiveness, sperm also covered the vertical substrates favored by many spawning females in our experiments. It is probable that as soon as a female spawns on the sperm-covered substrate, the oocytes can reactivate the inactive sperm (Fig. 7), and fertilization takes place. Experimentally, the best success (>75%) was achieved when the delay between the male and female spawnings was no more than 11 h; how-

ever, success was still good (>50%) after as long as 30 h (Fig. 8). A contact of 20–50 min with the oocyte seemed to be necessary for the spermatozoan to attain an optimum speed that probably maximizes its ability to fertilize. Sperm inactivity and reactivation appears to be very rare in marine habitats. Although sperm chemotaxis has been shown in echinoderms (Miller, 1985), no significant velocity increase or activation of the attracted sperm has ever been mentioned, except in cnidarians (Miller, 1979a, b) and larvaceans (Miller and King, 1983). The closest example with a similarity to *Leptasterias polaris* is the sperm of the horseshoe crab (*Limulus polyphemus*), which undergoes a brief flurry of motility and remains nonmotile until it encounters a sperm motility initiating molecule (SMI) emanating from eggs (Clapper and Epel, 1981). In contrast, both our observations and previous studies (Chia, 1968) show that the sperm of most echinoderms becomes active upon release in the water and disperses quickly. This is probably the best strategy when both male and female gametes are released in great numbers in the seawater at close intervals.

In *Leptasterias polaris,* the gamete behavior seems well adapted to the brooding mode, which in turn has a protective function. Brooded and nonbrooded embryos showed almost perfect synchrony in development through the gastrula stage (Table II). This suggests the absence of the obligatory exchange of nutrients between parent and young that is seen in *Pteraster militaris* (McClary and Mladenov, 1990), where a brood chamber is present. In *L. hexactis,* another brooding asteroid overlaying its eggs, the maternal presence is proposed to be essential to help the hatching embryo tear the fertilization membrane (Chia, 1966). Although this was thought to be the case for *L. polaris* (Himmelman *et al.,* 1982), we observed no evidence of that phenomenon. The hatching was not delayed and no loss of embryos was evident in the unbrooded group. Brooding in *L. polaris* probably serves mainly to aerate the embryos and prevent them from being covered with sediment as they lie on the substrate. Observations in the field (Himmelman *et al.,* 1982) support this hypothesis, as the substrate under a brooding starfish was always found clear of debris. Brood protection also seems to be in direct relation to adverse environmental conditions and predatory pressures. Extremely active grazers such as sea urchins, which are abundant wherever *L. polaris* is found, would rapidly decimate any unprotected starfish embryos exposed on a rock (Fig. 5a).

The embryonic development observed in *Leptasterias polaris* is similar to that described for *L. hexactis* (Chia, 1968) and *L. acceptances similispinis* (Kubo, 1951). The developmental kinetics of *L. polaris* is characteristically slower (Table I) than in all other reports for this genus, perhaps because of the lower temperatures (0°–1°C) found during the breeding and the subsequent development of this species. The major differences between our results and those of Kubo (1951) and Chia (1968) are the occurrence of a spinning stage and the much earlier hatching of *L. polaris.* Because *L. polaris* embryos hatch in late gastrula, before they develop brachiolar arms, such arms cannot contribute to the tearing of the fertilization membrane, as they are said to do in the two other species.

Table II

Leptasterias polaris: *Development of brooded and nonbrooded embryos*

	Brooded Embryos		Nonbrooded Embryos	
Developmental Stages	Time	Size (μm)	Time	Size (μm)
Fertilization	0	870 ± 43	0	852 ± 36
2-cell	43 h	1111 ± 22	46 h	1079 ± 15
4-cell	81 h	1092 ± 18	86 h	1032 ± 15
8-cell	93 h	1032 ± 9	92 h	1046 ± 12
16-cell	104 h	1044 ± 24	106 h	1032 ± 18
32-cell	125 h	1050 ± 33	121 h	1062 ± 9
64-cell	140 h	1082 ± 39	133 h	1103 ± 62
128-cell	152 h	1086 ± 41	146 h	1080 ± 3
256-cell	164 h	1092 ± 52	156 h	1101 ± 40
Blastula	8–9 d	1090 ± 62	8–9 d	1120 ± 51
Gastrula	20–21 d	1288 ± 52	20–21 d	1111 ± 33
Hatching	33–34 d	1121 ± 42	32–35 d	1199 ± 63

A new stage was considered attained when 50%–60% of the embryos reached it. The standard deviations about the mean size are given.

The freshly spawned eggs are negatively buoyant and do not adhere to one another until a few moments later, after fertilization. This stickiness was also observed by Chia (1968) for *Leptasterias hexactis,* but was correlated with a reaction to seawater rather than with fertilization. Through its growth, the embryo undergoes many changes in attachment capacity, which is first provided by the sticky fertilization membrane, then by small unciliated body areas after hatching, and later by the fixing disk and ramified tips of the brachiolar arms. The parental protection is probably useful in preventing dispersion of embryos during these changes in fixation ability, for instance during hatching, when attachment to the substrate may be ineffective for a short time. After the metamorphosis of the embryos (4–5 months), brooding individuals are still observed in the field for at least one month. The free-moving young starfish seem to remain under protection through the development of the pyloric caeca and the opening of the mouth. Environmental factors apparently play a role in the development of the embryos, especially in initiating metamorphosis by a considerable increase in temperature (Fig. 2). As previously mentioned by Boivin *et al.* (1986), this correlation seems to ensure that the young starfish are ready for release at the proper time, namely spring, when conditions are most favorable for their survival as

self-sufficient individuals. This timing control, together with the possibly lower energetic cost required from the parent under cold temperatures, is probably an advantage of winter brooding.

Acknowledgments

We thank Dr. C. Bouland for her help during the experiments, A. Caron for helpful criticism and assistance with statistical analysis, and J.-L. Théberges for photoprocessing. We greatly appreciated the helpful comments of Drs. S. Demers, J. H. Himmelman, C. Bouland, M. I. El-Sabh, and two anonymous reviewers on the manuscript. We are also grateful to J. Noël for her collaboration on the sketches and to N. Piché for photographs of *Leptasterias* and field observations. This work was carried out thanks to the space and material lent to us by Drs. É. Pelletier and F. Dubé at the Station Aquicole de Pointe-au-Père and was supported by personal funds of J.-F. Hamel and A. Mercier.

Literature Cited

Blankley, W. O., and G. M. Branch. 1984. Co-operative prey capture and unusual brooding habits of *Anasterias rupicola* (Verrill) (Asteroidea) at sub-Antarctic Marion Island. *Mar. Ecol. Prog. Ser.* **20:** 171–176.

Boivin, Y., D. Larrivée, and J. H. Himmelman. 1986. Reproductive cycle of the subarctic brooding asteroid *Leptasterias polaris*. *Mar. Biol.* **92:** 329–337.

Chia, F.-S. 1966. Brooding of a six-rayed starfish, *Leptasterias hexactis*. *Biol. Bull.* **130:** 304–305.

Chia, F.-S. 1968. The embryology of a brooding starfish, *Leptasterias hexactis* (Stimpson). *Acta Zool.* Bd XLIX: 1–44.

Chia, F.-S., and C. W. Walker. 1991. Echinodermata: Asteroidae. Pp. 301–353 in *Reproduction of Marine Invertebrates*. A. C. Giese, J. S. Pearse, and V. B. Pearse, eds. Boxwood Press, Pacific Grove, California.

Clapper, D. L., and D. Epel. 1981. Isolation and utilization of a sperm motility initiating peptide in the horseshoe crab *Limulus polyphemus*: evidence for involvement of Ca^{2+} but not intracellular pH or membrane potential in motility initiation. *J. Cell Biol.* **91:** 179.

Emerson, C. J. 1977. Larval development of the sea star, *Leptasterias polaris*, with particular reference to the optic cushion and ocelli. *Scan. Elec. Micro.* **2:** 631–638.

Giese, A. C., and H. Kanatani. 1987. Maturation and spawning. Pp. 251–313 in *Reproduction of Marine Invertebrates*, Vol. 9, A. C. Giese, J. S. Pearse, and V. B. Pearse, eds. Blackwell Scientific Publications, Palo Alto, California.

Giese, A. C., and J. S. Pearse. 1974. Introduction: general principles. Pp. 1–49 in *Reproduction of Marine Invertebrates*, Vol. 1, *Acoelomate and Pseudocoelomate Metazoans*. A. C. Giese and J. S. Pearse, eds. Academic Press, New York.

Gherardi, F., and M. Vannini. 1993. Hermit crabs in a mangrove swamp: proximate and ultimate factors in the clustering of *Clibanarius laevimanus*. *J. Exp. Mar. Biol. Ecol.* **168:** 167–187.

Hendler, G., and D. L. Meyer. 1982. Ophiuroids *flagrante delicto* and notes on the spawning behavior of other echinoderms in their natural habitat. *Bull. Mar. Sci.* **32:** 600–607.

Himmelman, J. H. 1981. Synchronization of spawning in marine invertebrates by phytoplankton. Pp. 3–19 in *Advances in Invertebrate Reproduction*. W. H. Clark, Jr. and T. S. Adams, eds. Elsevier North Holland, New York.

Himmelman, J. H., and C. Dutil. 1991. Distribution, population structure and feeding of subtidal seastars in the northern Gulf of St. Lawrence. *Mar. Ecol. Prog. Ser.* **76:** 61–72.

Himmelman, J. H., Y. Lavergne, A. Cardinal, G. Martel, and P. Jalbert. 1982. Brooding behaviour of the northern sea star *Leptasterias polaris*. *Mar. Biol.* **68:** 235–240.

Hyman, L. H. 1955. *The Invertebrates: Echinodermata*. McGraw-Hill, New York, 763 pp.

Kanatani, H., and H. Shirai. 1968. Does GSS or MIS act as a pheromone? Problems concerning the participation of a pheromone in starfish spawning. *Zool. Mag.* **77:** 207–212.

Komatsu, M. 1983. Development of the sea-star, *Archaster typicus*, with a note on male-on-female superposition. *Annot. Zool. JPN* **56:** 187–195.

Kubo, K. 1951. Some observations on the development of the sea-star, *Leptasterias acceptances similispinis* (Clarck). *J. Fac. Sci. Hokkaido Univ. Ser. VI, Zool.* **10:** 97–105.

Levitan, D. R. 1988. Asynchronous spawning and aggregative behavior in the sea urchin *Diadema antillarum* (Philippi). Pp. 181–186 in *Echinoderm Biology*. R. D. Burke, P. V. Mladenov, P. Lambert, and R. L. Parsley, eds. Balkema, Rotterdam.

Levitan, D. R. 1991. Influence of body size and population density on fertilization success and reproductive output in a free-spawning invertebrate. *Biol. Bull.* **181:** 261–268.

Levitan, D. R., M. A. Sewell, and F.-S. Chia. 1992. How distribution and abundance influence fertilization success in the sea urchin *Strongylocentrotus franciscanus*. *Ecology* **73:** 248–254.

Lewis, J. B. 1958. The biology of the tropical sea urchin *Tripneustes esculentus* Leske in Barbados, West Indies. *Can. J. Zool.* **36:** 607–621.

McClary, D. J., and P. V. Mladenov. 1990. Brooding biology of the sea star *Pteraster militaris* (O. F. Müller): energetic and histological evidence for nutrient translocation to brooded juveniles. *J. Exp. Mar. Biol. Ecol.* **142:** 183–199.

McEuen, F. S. 1988. Spawning behaviors of northeast Pacific sea cucumbers (Holothuroidea: Echinodermata). *Mar. Biol.* **98:** 565–585.

Mercier, A., É. Pelletier, and J.-F. Hamel. 1994. Metabolism and subtle toxic effects of butyltin compounds in starfish. *Aquat. Toxicol.* **28:** 259–273.

Miller, R. L. 1979a. Sperm chemotaxis in the Hydromedusae. I. Species-specificity and sperm behavior. *Mar. Biol.* **53:** 99–114.

Miller, R. L. 1979b. Sperm chemotaxis in the Hydromedusae. II. Some chemical properties of sperm attractants. *Mar. Biol.* **53:** 115–124.

Miller, R. L. 1985. Demonstration of sperm chemotaxis in Echinodermata: Asteroidea, Holothuroidea, Ophiuroidea. *J. Exp. Zool.* **234:** 383–414.

Miller, R. L. 1989. Evidence for the presence of sexual pheromones in free-spawning starfish. *J. Exp. Mar. Biol. Ecol.* **130:** 205–221.

Miller, R. L., and K. King. 1983. Sperm chemotaxis in *Oikopleura dioica* (Urochordata: Larvaceae). *Biol. Bull.* **165:** 419–428.

Minchin, D. 1987. Sea-water temperature and spawning behaviour in the seastar *Marthasterias glacialis*. *Mar. Biol.* **95:** 139–143.

O'Brien, F. X. 1976. Some adaptations of the seastar, *Leptasterias* (Stimpson) to life in the intertidal zone. *Thalassia Jugosl.* **12:** 237–243.

Ohshima, H., and H. Ikeda. 1934. Male-female superposition of the sea-star *Archaster typicus* Müller et Troschel. *Zool. Lab. Kyushu Imperial University* **54:** 125–128.

Ormond, R. F. G., A. C. Campbell, S. H. Head, R. J. Moore, P. R. Rainbow, and A. P. Saunders. 1973. Formation and breakdown of aggregations of the crown-of-thorns starfish, *Acanthaster planci* (L.). *Nature* **246:** 167–169.

Orton, J. H. 1914. On the breeding habit of *Echinus miliaris*, with note on the feeding habits of *Patella vulgata*. *J. Mar. Biol. Ass. U. K.* **10:** 254–257.

Pain, S. L., P. A. Tyler, and J. D. Gage. 1982. The reproductive biology of *Hymenaster membranaceus* from the Rockall Trough, North East Atlantic Ocean, with notes on *H. gennaeus*. *Mar. Biol.* **70:** 41–50.

Pearse, J. S., and R. A. Cameron. 1991. Echinodermata: Echinoidea. Pp. 513–662 in *Reproduction of Marine Invertebrates: Lophophorates, Echinoderms*. Vol. VI, A. C. Giese, J. S. Pearse, and V. B. Pearse, eds. Boxwood Press, Pacific Grove, California.

Pearse, J. S., and C. W. Walker. 1986. Photoperiodic regulation of gametogenesis in a North Atlantic sea star *Asterias vulgaris*. *Int. J. Invert. Reprod. Develop.* **9:** 71–77.

Pearse, J. S., D. J. Eernisse, V. B. Pearse, and K. A. Beauchamp. 1986. Photoperiodic regulation of gametogenesis in sea stars, with evidence for an annual calendar independent of fixed daylength. *Am. Zool.* **26:** 417–431.

Pearse, J. S., D. J. McClary, M. A. Sewell, W. C. Austin, A. Perez-Ruzafa, and M. Byrne. 1988. Simultaneous spawning of six species of echinoderms in Barkley Sound, British Columbia. *Int. J. Invert. Reprod. Develop.* **14:** 279–288.

Pennington, J. T. 1985. The ecology of fertilization of echinoid eggs: the consequences of sperm dilution, adult aggregation and synchronous spawning. *Biol. Bull.* **169:** 417–430.

Run, J.-Q., C.-P. Chen, K.-H. Chang, and F.-S. Chia. 1988. Mating behaviour and reproductive cycle of *Archaster typicus* (Echinodermata: Asteroidea). *Mar. Biol.* **99:** 247–253.

Slattery, M., and I. Bosch. 1993. Mating behavior of a brooding Antarctic asteroid, *Neosmilaster georgianus*. *Int. J. Invert. Reprod. Develop.* **24:** 97–102.

Sloan, N. A. 1980. Aspects of the feeding biology of asteroids. *Oceanogr. Mar. Biol. Ann. Rev.* **18:** 57–124.

Sloan, N. A. 1984. Interference and aggregation: close encounters of the starfish kind. *Ophelia* **23:** 23–31.

Sloan, N. A., and A. C. Campbell. 1982. Perception of food. Pp. 3–23 in *Echinoderm Nutrition*. M. Jangoux and J. M. Lawrence, eds. A. A. Balkema, Rotterdam.

Starr, M., J. H. Himmelman, and J.-C. Therriault. 1990. Direct coupling of marine invertebrate spawning with phytoplankton blooms. *Science* **247:** 1071–1074.

Strathmann, R. R. 1987. Evolutionary aspects of marine invertebrate reproduction. Pp. 609–665 in *Reproduction of Marine Invertebrates*. Vol. 9, A. C. Giese, J. S. Pearse, and V. B. Pearse, eds. Blackwell Scientific Publ., Palo Alto, and Boxwood Press, Pacific Grove, California.

Suzuki, N., M. Hoshi, K. Nomura, and S. Isaka. 1982. Respiratory stimulation of sea urchin spermatozoa by egg extracts, egg jelly extracts and egg jelly peptides from various species of sea urchins: taxonomical significance. *Comp. Biochem. Physiol.* **72:** 489–495.

Thorson, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.* **25:** 387–397.

Warner, G. F. 1979. Aggregation in echinoderms. Pp. 375–396 in *Biology and Systematics of Colonial Organisms*. G. Larwood and B. R. Rosen, eds. Academic Press, London.

Young, C. M., P. A. Tyler, J. L. Cameron, and S. G. Rumrill. 1992. Seasonal breeding aggregations in low-density populations of the bathyal echinoid *Stylocidaris lineata*. *Mar. Biol.* **113:** 603–612.

Yund, P. O. 1990. An *in situ* measurement of sperm dispersal in a colonial marine hydroid. *J. Exp. Zool.* **253:** 102–106.